Chapter 2

General characteristics of VHT-treated mango under low temperature storage

2.1 Introduction

Mango is the third largest cultivated fruit in the world but less than 10% of the total world production is exported. In Japan, the import of fresh mango fruit has increased continuously and these are mainly from The Philippines (cv.Carabao), Mexico (cv.Tomy Atkins, Haden), Thailand (Nang Klang Wan, Nam Dok Mai, Pimsen Dang, Rad) and Australia (cv. Kensington). Recently, in the Japanese market there have been about 6,000 tons of imported mangoes from The Philippines annually. This represents 68% of total as of 1999, and was followed by Mexico (27%), Thailand (2%), Australia (1.6%), USA (1.3%) and Taiwan (0.4%).

In 1997, Thailand exported 8,522 tons of fresh mango worth US $ 4,025,378 (Arthachinta, 2000). However, the amount of export from Thailand to the Japanese market is small, only 107 tons in 1996. A short shelf life and susceptibility to postharvest decay and chilling injury limit its export to the distant markets. Improving quality is an urgent requirement to compete in world markets. The information from imported mango in Japan, such as the 'Carabao'

from The Philippines and the 'Nam Dok Mai' from Thailand, was collected in order to determine their physiological and biochemical changes such as respiration rate, ethylene production, color development, sugar and acid changes, and the incidence of chilling injury of the fruits stored at different temperatures which affect the postharvest behaviors of these fruit.

2.2 Experiment 1. Effect of low temperature storage on 'Carabao' mango after VHT.

2.2.1 Materials and Methods

Fruits

Mango fruits with different skin color stages were purchased from a wholesale market in Tsuchiura city: stage 3 = more green than yellow and stage 4 = more yellow than green (color index source: Mendoza and Wills (1984)) (Fig. 2-1).

Storage condition

Fruit of each stage were divided into 3 groups and each group was maintained at 5°C, 13 °C and 20 °C, respectively. The fruits were sampled for physiological and biochemical analysis at 4-day intervals. Skin and pulp color were measured by a color difference meter (Hunter color difference meter, model CR-100, Minolta), flesh firmness by a rheometer (type NRM-202J) using a 7

mm Ø plunger, total soluble solid (TSS) by a hand refractometer and titratable acid (TA) by titrating 10 ml of extracted juice with 0.1 NaOH and expressed as percent citric acid. Chilling injury was expressed as a rating by the appearance of severity of symptom as follows: 1 = none, 2 =slight, 3 =moderate and 4 =severe. CO_2 and C_2H_4 were measured by sealing the fruit samples in a 1- liter volume container for 3 hr. at 24 °C, then one ml of gas sample was withdrawn with a tight hypodermic syringe and analyzed by a gas chromatograph (HITACHI model 163 for ethylene and a Shimadzu GC-8A for carbon dioxide) under the following analysis conditions: column for carbon dioxide, WG-100; column temperature, 50 °C; carrier gas, Helium with 30 ml/min; detector, GCD with 120 mA current; and column for ethylene, Porapack Q; column temperature, 75 °C; carrier gas, N_2 with 20 ml/min; detector, FID. For each treatment, 3 gas samples were analyzed, respectively.

2.2.2 Results

Chilling injury and disorders

No internal and external symptoms of chilling injury appeared until the storage time reached the 14th day at storage temperatures. The fruits ripened normally when stored at 5 and 13 °C for 4 days, but fruits stored at 20 °C showed an expression of anthracnose infection which appeared as dark brown, circular and

sunken spots and some fruits showed symptoms of stem end rot; softening and water-soaked lesion were found at the infected tissue (Fig. 2-2).

Respiration rate

The CO_2 production of mango fruit from all treatments during the storage for 24 days is illustrated in Fig. 2-3. When stored at 20 °C, stage 3 and stage 4 fruits showed a higher CO_2 production than those stored at 5 and 13 °C. The respiration rate of the fruit at stage 3 increased rapidly and showed a climacteric peak on the 8th day, then decreased when fruit senescence occurred, whereas stage 4 fruits reached the peak 4 days earlier. When stored at 5 and 13 °C the respiration rate of the fruits at both stages remained steadily low during storage. This data indicated that stage 3 fruit have a longer storage life than stage 4 fruit.

Ethylene production

Fig. 2-4 shows the rapid increase of ethylene concentration of the fruit stored at 20 °C, reaching a peak at 8 days and 12 days for stage 4 and 3 fruits, respectively. When stored at 13 °C, stage 4 fruit moderately increased ethylene concentration with a peak on the 8th day, whereas stage 3 fruit evolved only a very low concentration of ethylene. No difference in ethylene evaluation was observed between both stages when stored at 5 °C as the ethylene

concentration was very low.

Total soluble solids (TSS)

The TSS of all the treatments tended to generally increase. Slower changes in TSS were observed when stored at the lower temperatures as compared with 20 °C (Fig.2-5).

Titratable acidity (TA)

A rapid decrease in titratable acidity was observed during the first 12 days in the case of both stage 3 and 4 fruits stored at 20 °C, while a much slower decrease of acidity was found in the fruits maintained at 13 °C and even slower at 5 °C (Fig. 2-6).

Weight loss

The rate in weight loss during storage increased with a similar tendency for both stage 3 and stage 4 fruits. When stored at 20 °C, it increased more rapidly than at 5 °C and 13 °C. In comparison with stage 3 fruit, the weight loss of stage 4 fruit was hastened when kept at 5 °C and 13 °C. Stage 4 fruits showed slightly faster weight loss when stored at 5 °C and 13 °C with no difference between both temperatures (Fig. 2-7).

2.3 Experiment 2. Low temperature storage and chilling injury of mango 'Nam Dok Mai' after VHT.

2.3.1 Materials and Methods

Fruits

Imported mature green mango 'Nam Dok Mai' fruits from Thailand were obtained from a trading company in Tokyo.

Storage condition

Fruits were divided into 3 groups and each group was maintained at 5°C, 13 °C and 20 °C, respectively. At 4 days interval, the fruits were sampled after storage and allowed to ripen at room temperature (20 °C). The sampled fruits were analyzed for physiological and biochemical changes by the same methods as described in 2.2.1.

2.3.2 Results

Chilling injury

The symptom of chilling injury appeared after storage for 8 days at 5 °C (Fig. 2-8) as indicated by abnormal ripening. The longer the time of storage, the more severity of chilling injury (Table 2-1) was found, showing skin browning, abnormal ripening and high susceptibility to diseases (Fig. 2-9).

Respiration rate

The CO_2 production of 'Nam Dok Mai' mango stored at

different temperatures varied; that of mango kept at 20 °C increased rapidly and reached the highest peak on the 8th day as a typical climacteric pattern, and then decreased. That at 5 and 13 °C was constantly low during storage (Fig. 2-10).

Ethylene production

When stored at 20 °C, fruits produced higher C_2H_4 concentration than at 5 and 13 °C with the peak on the 12th day, while ethylene concentration remained very low when stored at 5 and 13 °C. (Fig. 2-11).

Total soluble solids (TSS)

As shown in Fig. 2-12, TSS tended to increase during storage. The fruits at 20 °C increased TSS rapidly reaching the highest peak on the 4th day in storage and then gradually decreased as the fruits became over-ripe.

Titratable acidity (TA)

Despite a decrease in titratable acidity during storage time, the fruit maintained at 5 and 13 °C, had a relatively slower decrease in acidity. That at 20 °C was conspicuously similar to the pattern shown in 'Carabao' (Data not shown)

Weight loss

When stored at 20 °C, weight loss increased rapidly during the first 8 days, while that of fruit at 5 °C was quite low and stable. At 13 °C, weight loss increased until the 8th days similar to that at 20 °C, and then constant until 12 days and increasing rapidly again thereafter (Fig. 2-13).

2.4 Ultrastructural changes during chilling injury observed by scanning electron microscope (SEM)

2.4.1 Materials and Methods

Mature green mango 'Nam Dok Mai' fruits were stored at 5 °C and also at 20 °C as the control. After storage, fruits were transferred to room temperature for observation of chilling injury symptoms. Then mesocarp tissue was taken for microscopic examination. Thin specimens of damaged areas were excised and immediately immersed in 3% glutaraldehyde in a phosphate buffer (pH 7) for 12 hr. The tissue was postfixed in 1% osmium tetraoxide for 1 hr., and then dehydrated in a graded series of alcohol and acetone. After drying at the critical point, it was mounted on aluminum stubs and spatter-coated with gold before viewed by a scanning electron microscope (SEM) (Model S430 Hitachi).

2.4.2 Results

Under SEM observation, the control fruits at 2 weeks after storage showed that the mesocarp consisted of normal parenchyma cells with uniform cell walls and no starch granules in the cells (Fig. 2-14a).

The chilling treated samples in Fig. 2-14b and Fig. 2-15b after storage showed that cell walls were not uniform in thickness and starch granules were still in the parenchyma cells similar to the fruits before storage (Fig. 2-15a). The presence of starch granule was an indication of abnormal ripening when chilling injury occurred. However, in this study chilling injured fruits showed neither evidence of separation of cell walls nor collapsed mesocarp cells.

2.5 Discussion

The results showed that 'Carabao' mango of stage 3 had longer storage life compared with stage 4 fruits because they delayed in the ripening process (based on C_2H_4 production, respiration rate and skin color changes). The fruits stored at 5 °C showed only a slight internal breakdown symptom (spongy tissue with starchy appearance) when ripened at ambient temperature. In 'Carabao' mango the susceptibility to internal breakdown and chilling injury (CI) is affected by maturity and source of fruit.

However the fruits used in this experiment did not show CI. Chaplin et al. (1984) proposed that pre-conditioning by a step-wise reduction of temperature could be used to increase resistance to CI. In this case 'Carabao' mango subjected to VHT for fruit fly control for export may decrease susceptibility to CI at 5 °C.

The results from Experiment 2 showed that the ripening pattern of 'Nam Dok Mai' mango in terms of firmness, TA, TSS and skin color changed slower at 5 °C than at 13 °C. The first symptom of chilling injury (abnormal ripening and disease) was detected in 'Nam Dok Mai' stored at 5 °C for 16 days and then ripened at 20 °C. This was confirmed by the observation using a scanning electron microscope (SEM) that parenchyma cells of chilled fruits contained a large number of starch grains when stored at 5 °C for 2 weeks. Thus, the process for metabolism of starch to sugar during ripening was inhibited as was found in heat-injured fruits of 'Kensington' mango (Jacobi and Gowanlock, 1995). However, the physiological disorder such as internal breakdown was not expressed.

From both experiments, the critical temperature for being susceptible to CI may depend on the cultivars. 'Carabao' mango from The Philippines showed a higher tolerance at lower temperature in storage when compared with 'Nam Dok Mai' mango.

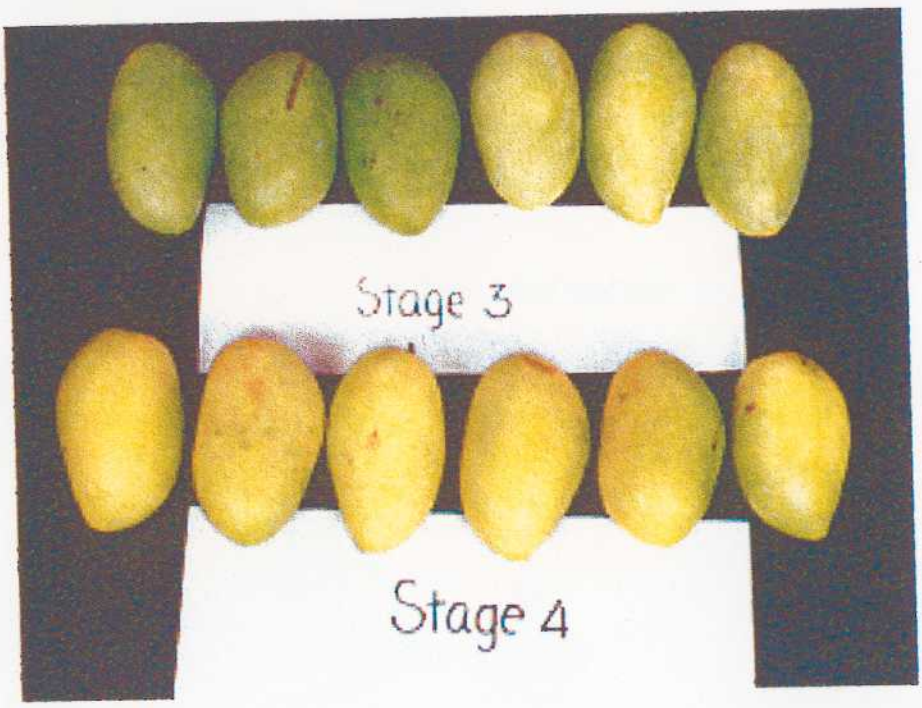

Fig. 2-1. Skin colors of 'Carabao' mango by using color index (stage 3: more green than yellow and stage 4: more yellow than green).

Fig. 2-2. The 'Carabao' mango fruit stored at different temperatures for 4 and 14 days.

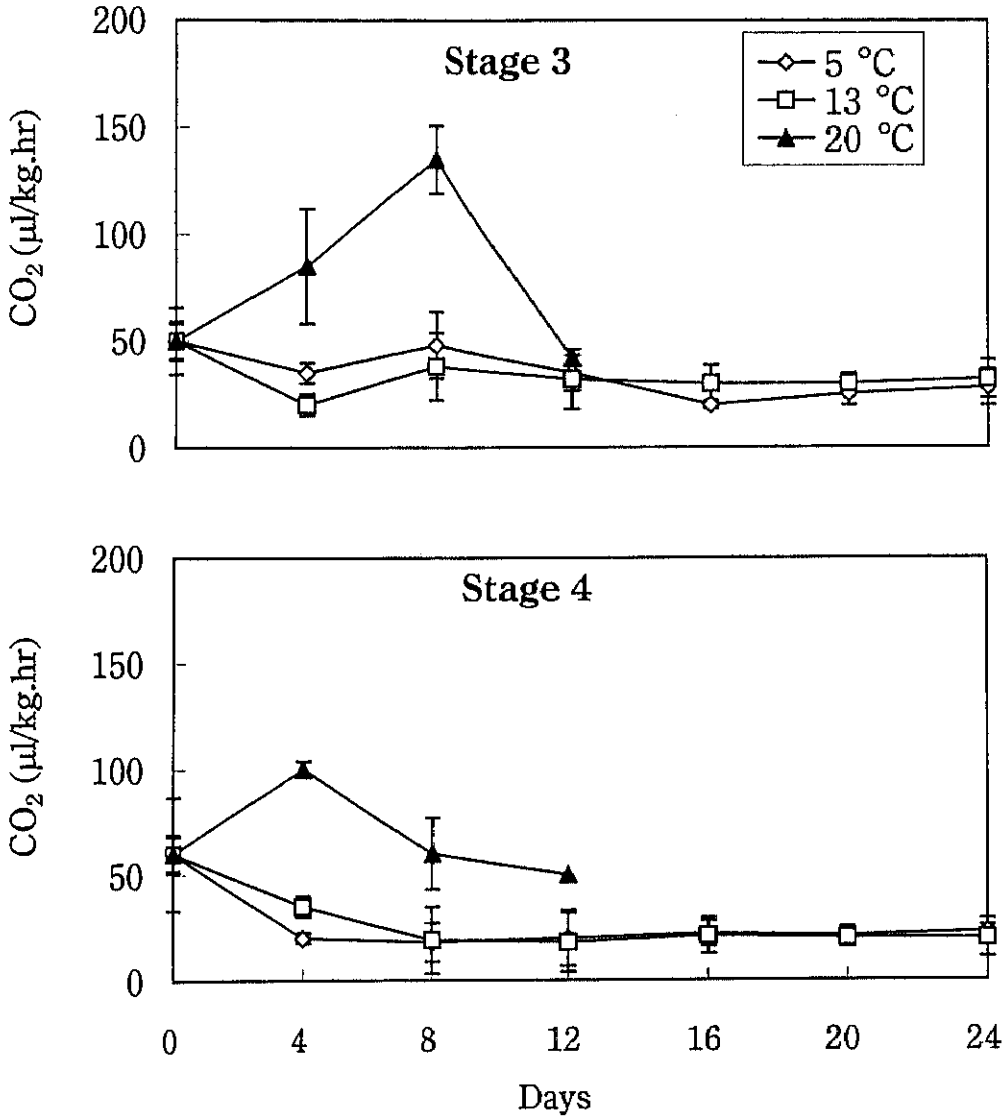

Fig. 2-3. Changes in respiration of 'Carabao' mango fruits with different ripening stages and temperatures.

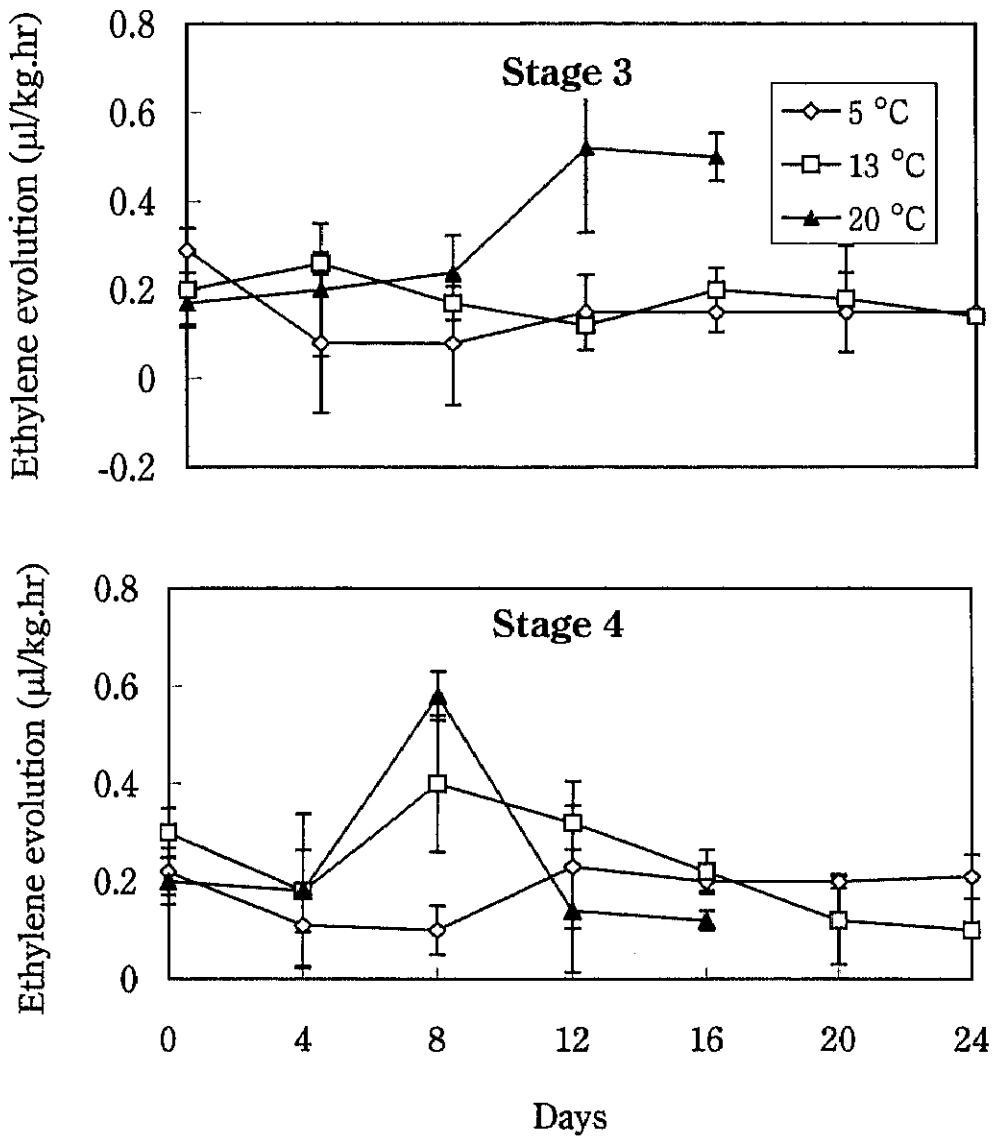

Fig. 2-4. Changes in ethylene evolution of 'Carabao' mango fruits with different ripening stages and temperatures.

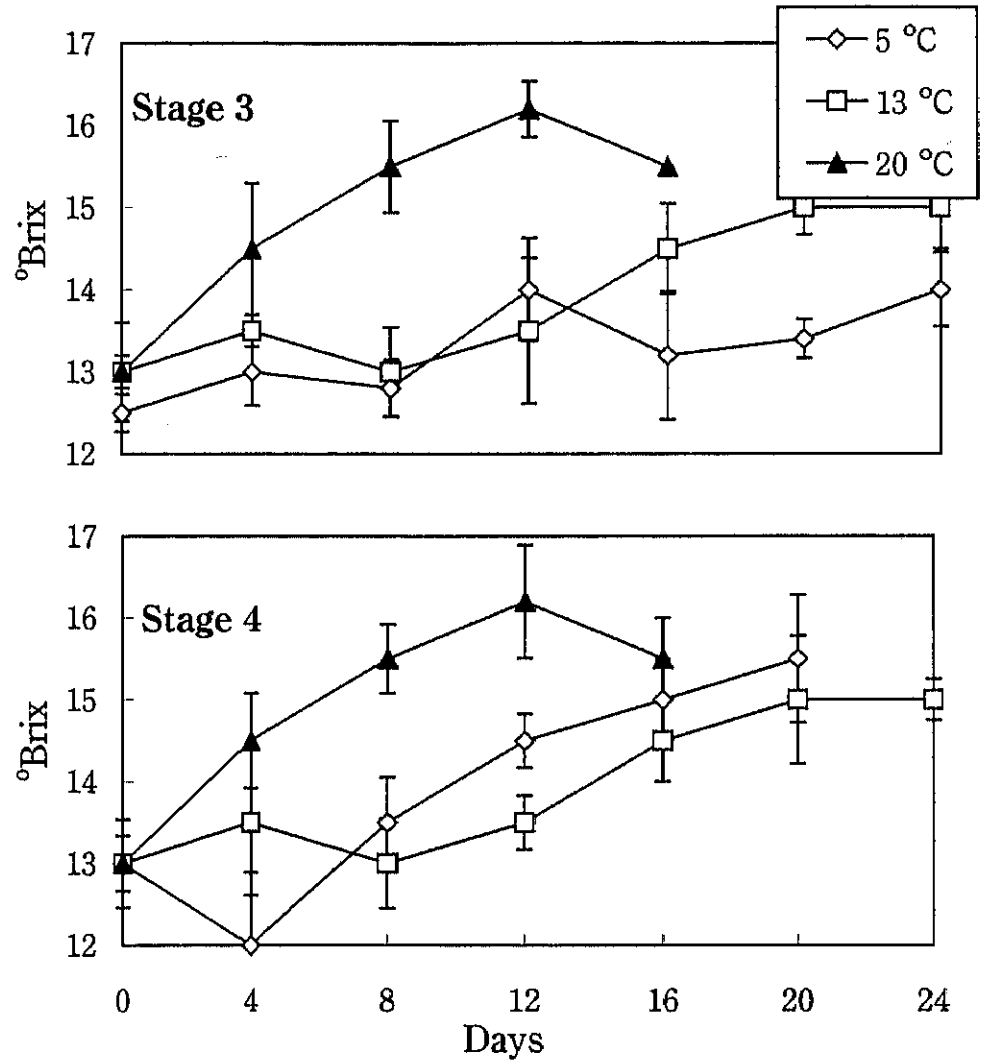

Fig. 2-5. Changes in total soluble solids (TSS) of 'Carabao' mango fruits with different ripening stages and temperatures.

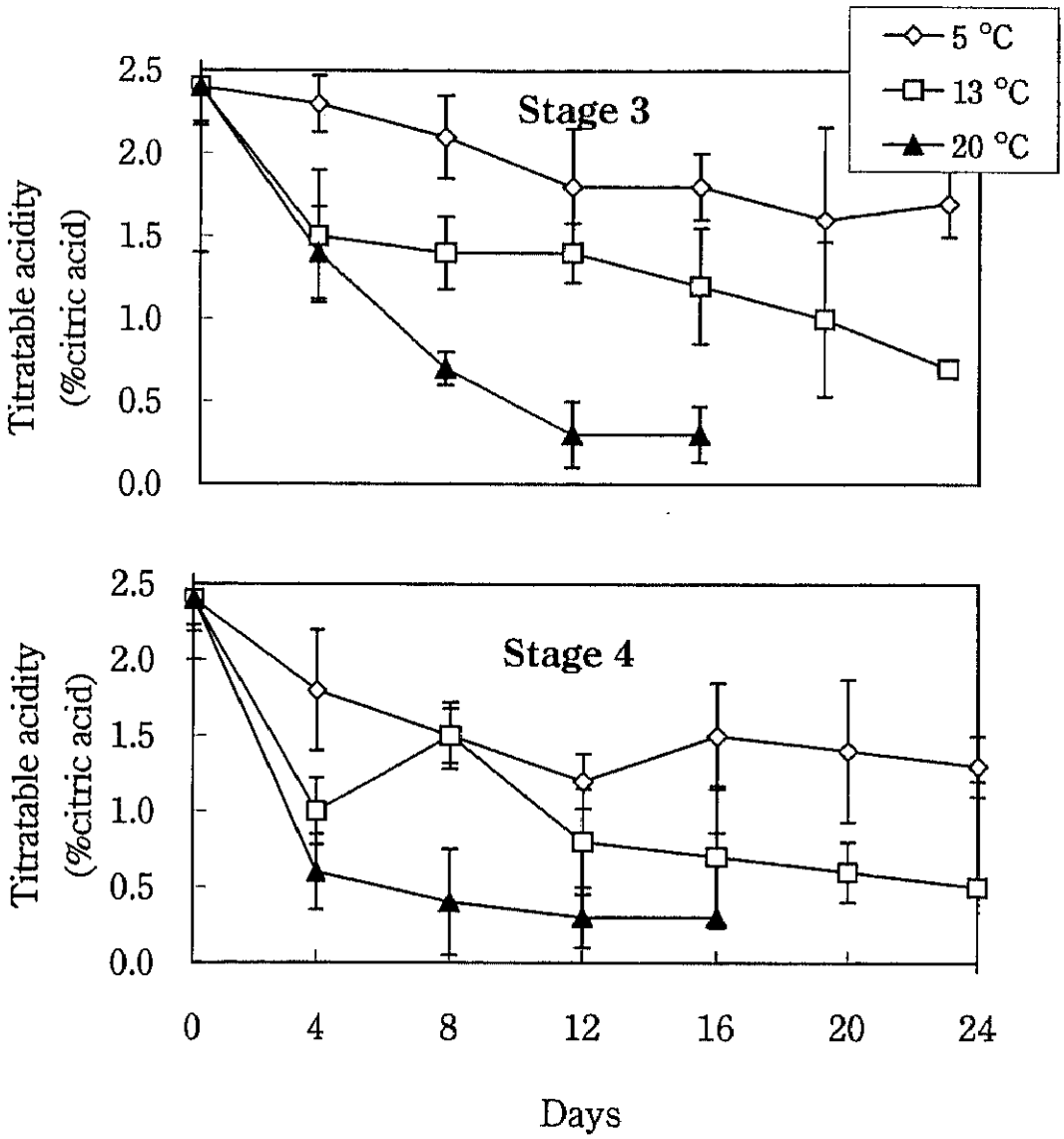

Fig. 2-6. Changes in titratable acidity (TA) of 'Carabao' mango fruits with different ripening stages and temperatures.

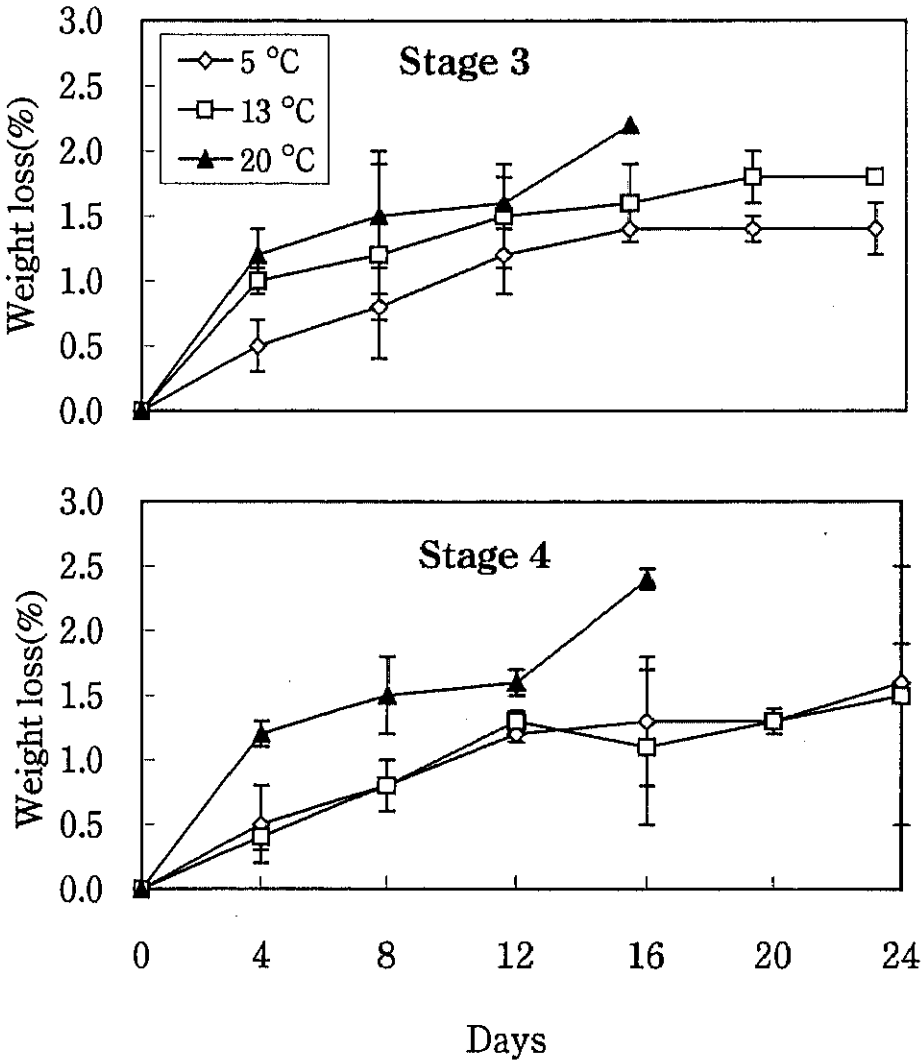

Fig. 2-7. Changes in weight loss (%) of 'Carabao' mango fruits with different ripening stages and temperatures.

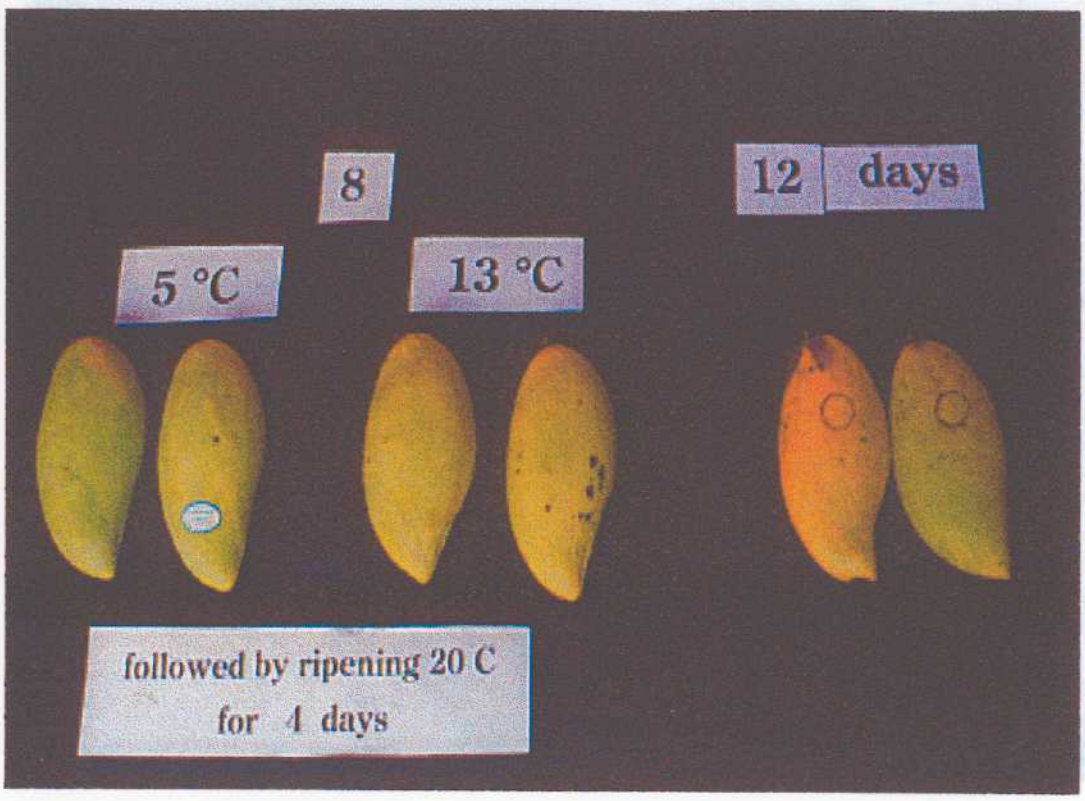

Fig. 2-8. 'Nam Dok Mai' mango fruits when stored at 5 °C (left), 13 °C (center) for 8 days and transferred to ripen at 20 °C, and fruits stored at 20 °C for 12 days (right).

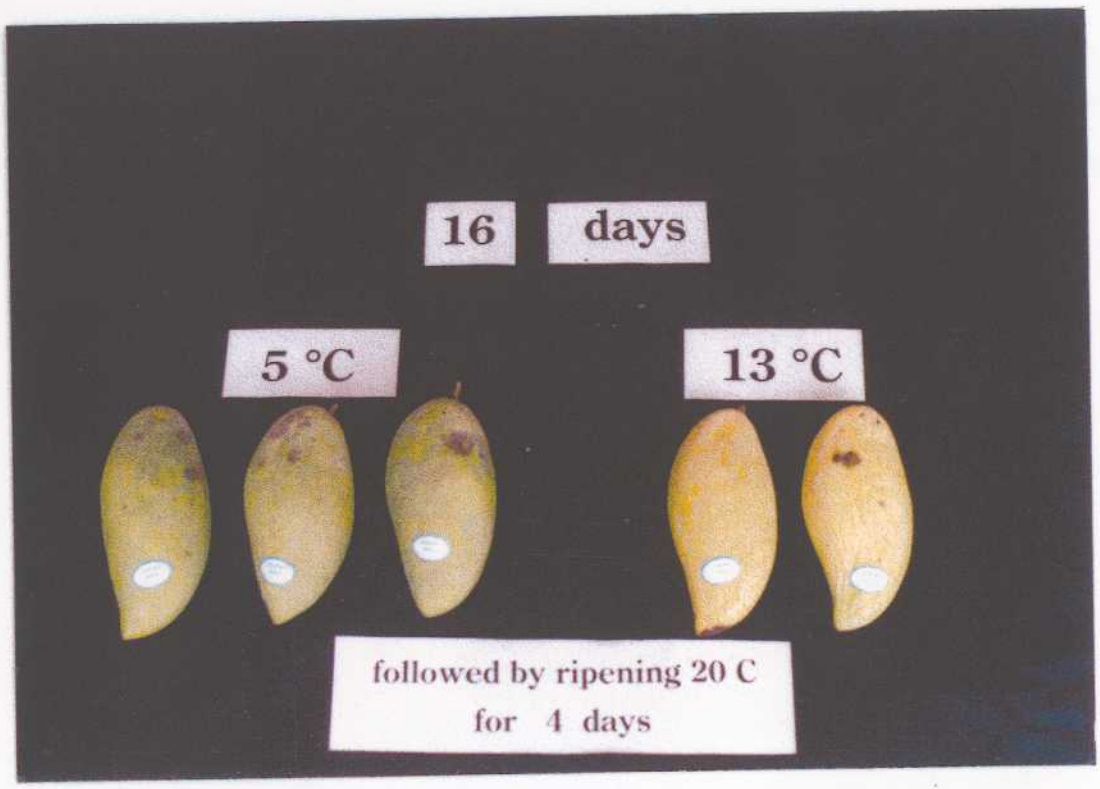

Fig. 2-9. Chilling injury symptoms of 'Nam Dok Mai' mango after stored at 5°C for 16 days.

Table 2-1. Chilling injury symptoms of imported 'Nam Dok Mai' mango during storage at 5, 13 and 20 °C.

Temperature (°C)	Storage time (days)	Ripening (days)	CI symptom		Disease	Abnormal ripening
			Skin	Pulp browning		
5	4	4	-	-	2	+ *
	8	4	-	-	3	++
	16	4	3**	-	3	+++
	32	4	4	-	4	++++
13	4	4	-	-	-	-
	8	4	2	-	2	-
	16	4	2	-	3	-
	32	-	-	-	-	-
20	4	-	-	-	-	-
	8	-	-	-	-	-

*Abnormal ripening value was assessed on a degree of the visual appearance of ripening from +(normal) to ++++ (abnormal).

**CI symptom was rated on a scale of 0 (no CI symptom) to 4 (severe).

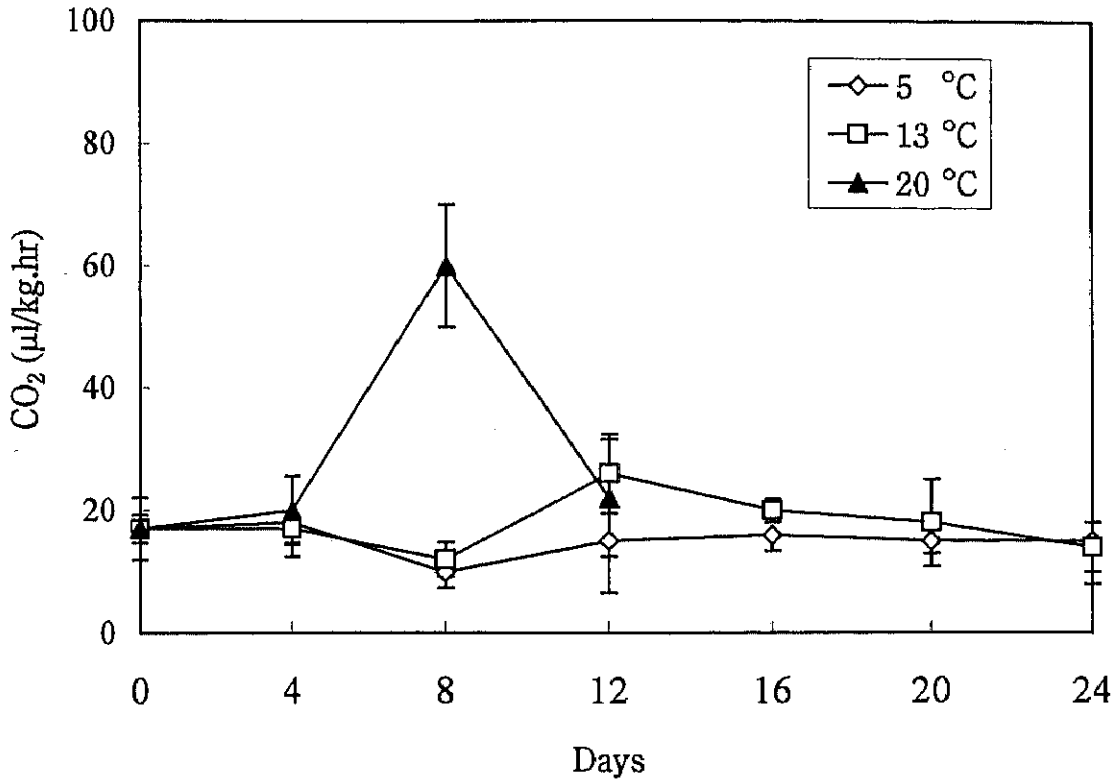

Fig. 2-10. Changes in respiration rate of 'Nam Dok Mai' mango fruits when stored at different temperatures.

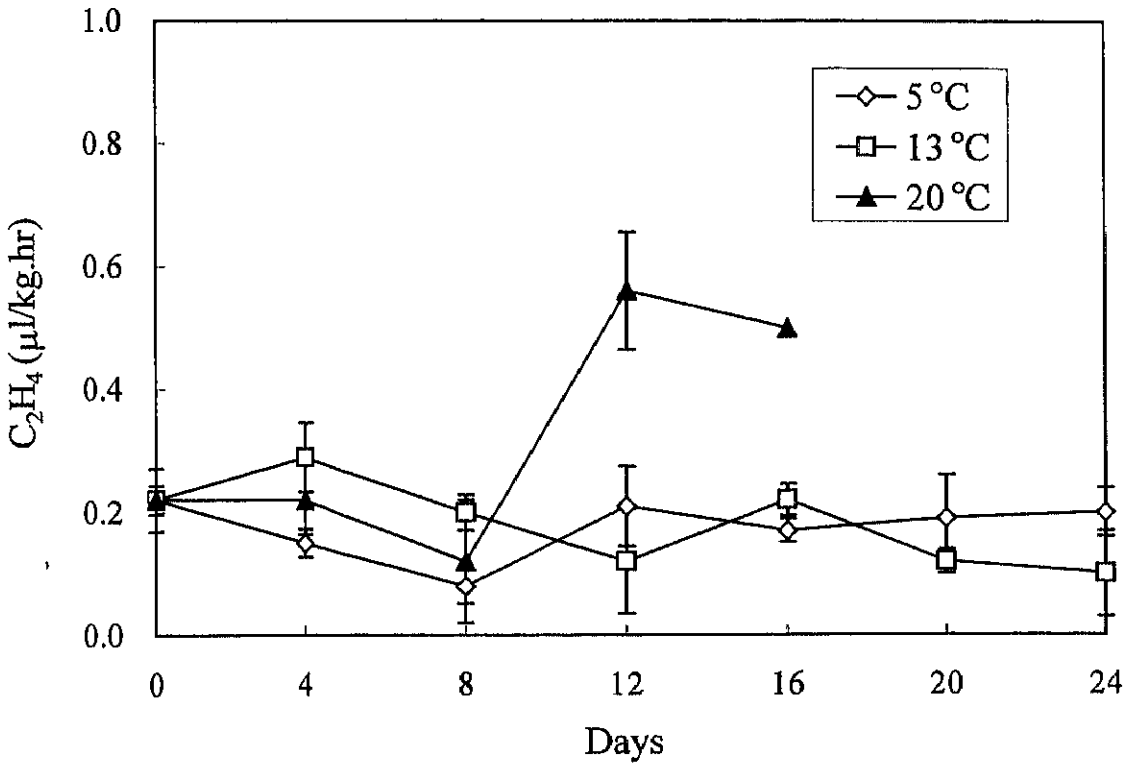

Fig. 2-11. Changes in ethylene evolution of 'Nam Dok Mai' mango fruits when stored at different temperatures.

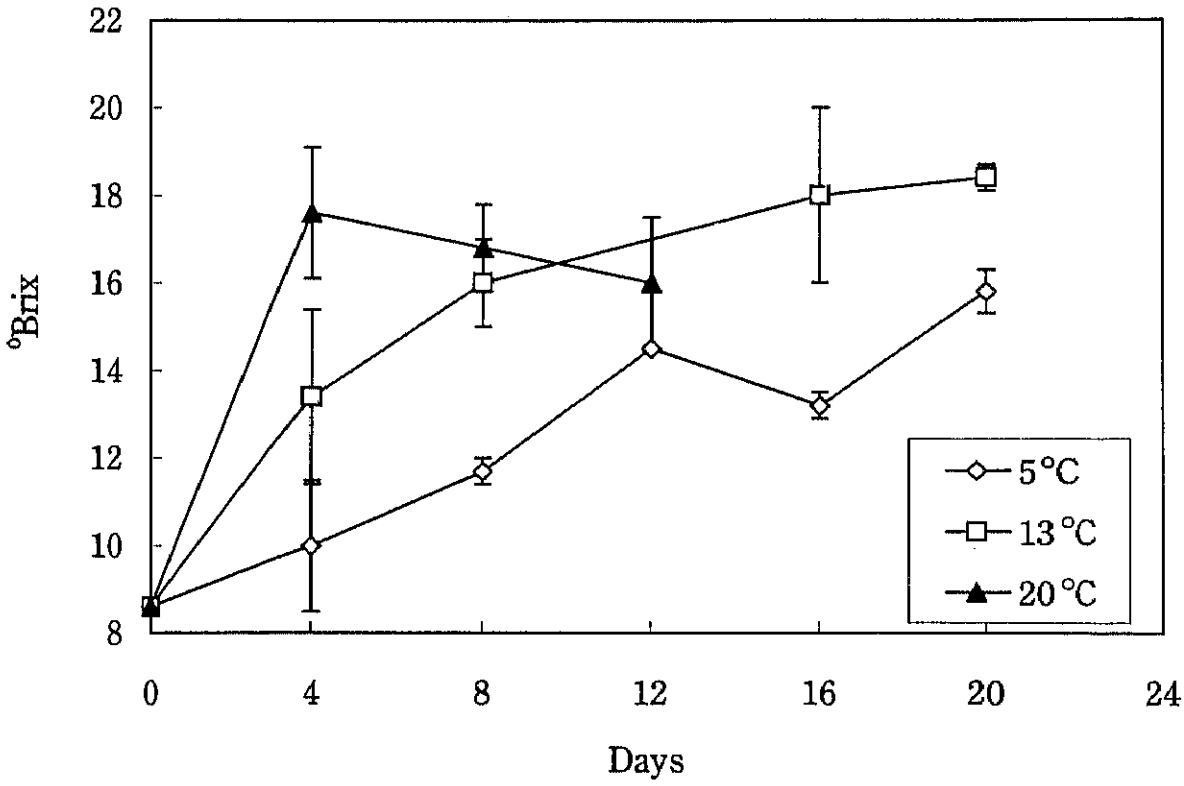

Fig. 2-12. Changes in total soluble solids (TSS) of 'Nam Dok Mai' mango fruits when stored at different temperatures.

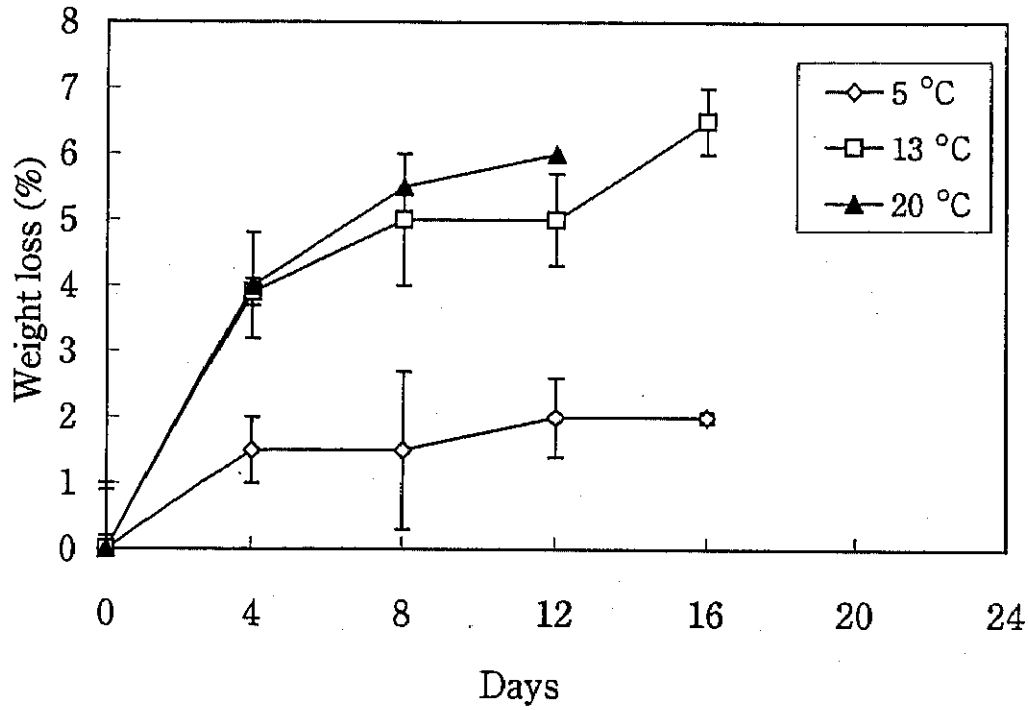

Fig. 2-13. Changes in weight loss of 'Nam Dok Mai' mango fruits when stored at different temperatures.

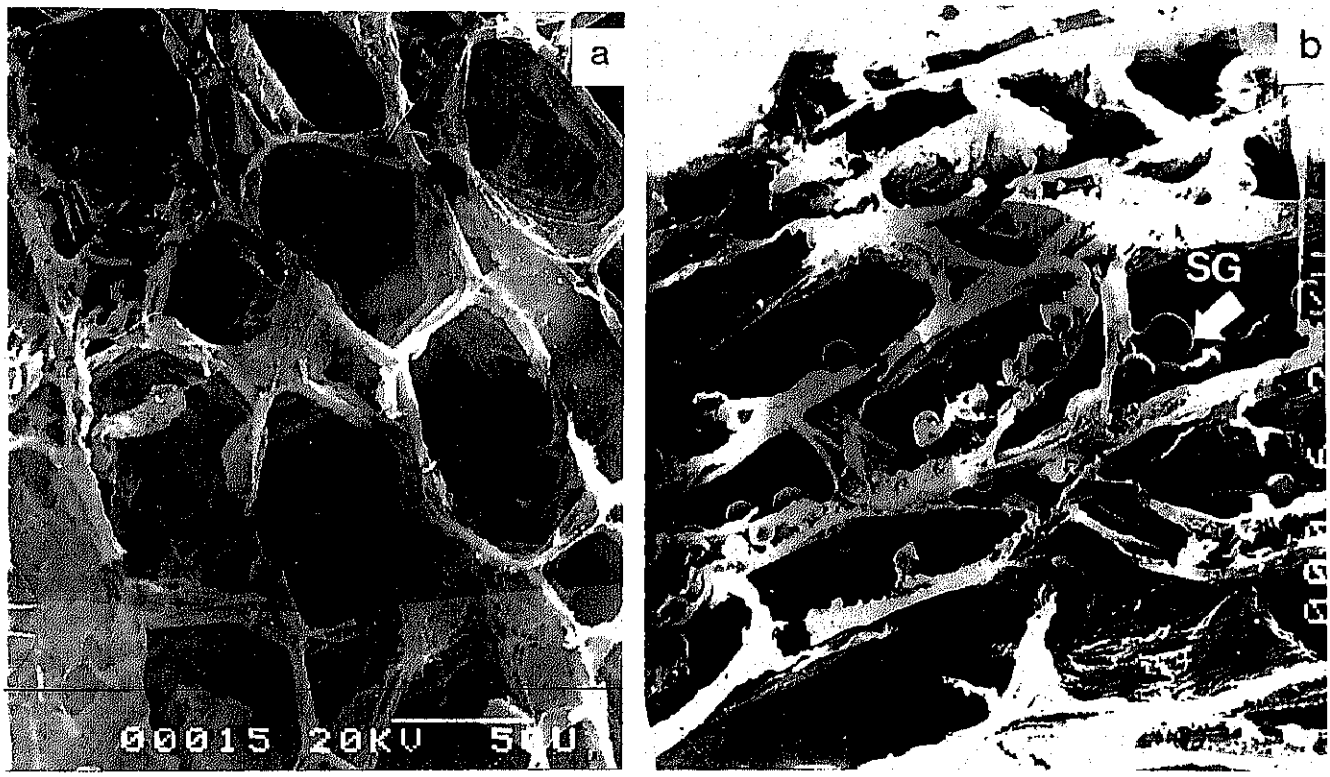

Fig. 2-14. Scanning electron micrographs of (a) ripe non-chilled fruit and (b) chilled 'Carabao' mango fruit after storage at 5°C for 2 weeks. Note : chilled tissue showed the parenchyma cell containing starch grains. SG=starch grain.

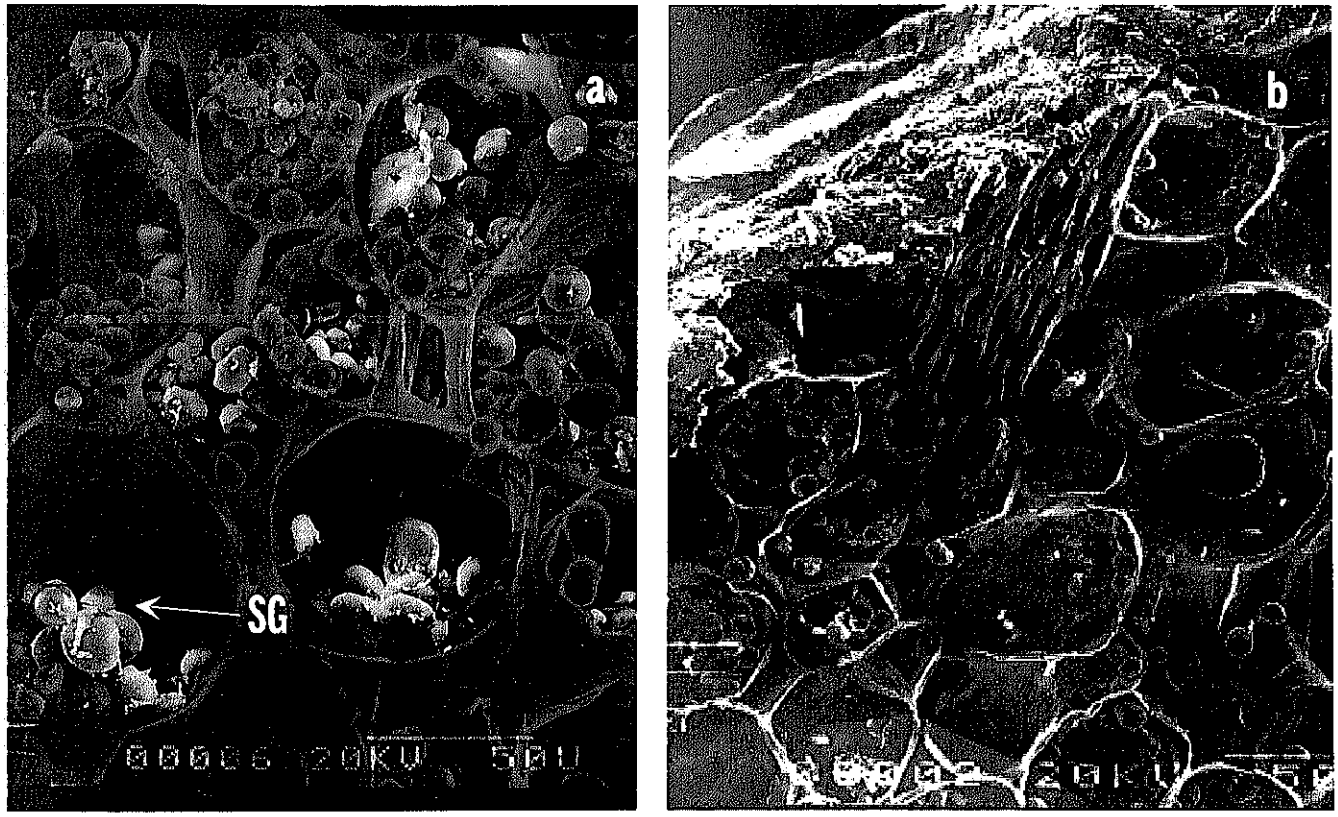

Fig. 2-15. Scanning electron micrographs of unripe 'Nam Dok Mai' mango fruit (a) before storage and (b) after storage at 5°C for 2 weeks showing the parenchyma cells containing starch grains.